Journal of Toxicology and Environmental Health

ISSN: 0098-4108 (Print) (Online) Journal homepage: http://www.tandfonline.com/loi/uteh19

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To cite this article: Robert C. Wong & Jeffrey B. Stevens (1986) Bipyridylium herbicide toxicity in vitro: Comparative study of the cytotoxicity of paraquat and diquat toward the pulmonary alveolar macrophage, Journal of Toxicology and Environmental Health, 18:3, 393-407, DOI: 10.1080/15287398609530880

To link to this article: http://dx.doi.org/10.1080/15287398609530880

Published online: 20 Oct 2009.

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BIPYRIDYLIUM HERBICIDE TOXICITY IN VITRO: COMPARATIVE STUDY OF THE CYTOTOXICITY OF PARAQUAT AND DIQUAT TOWARD THE PULMONARY ALVEOLAR MACROPHAGE

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In vitro exposure of adult rat alveolar macrophages to either paraquat or diquat resulted in concentration dependent cytotoxicity (cell death). The herbicide paraquat, however was statistically significantly more potent toward these cells than was diquat. The LC50 value for paraquat (8-h exposure, 37°C) was determined to be 0.94 mM [95% confidence interval (C.I.) 0.79–1.12 mM], whereas the corresponding LC50 value for diquat was 1.97 mM (C.I. 1.58–2.51 mM).

Interestingly, diquat was shown to enter these cells to a much greater extent than was paraquat. The latter data, while seemingly contradictory to the above findings, is consistent with other reported findings in this study that show that cell respiration, as measured by loss of oxygen consumption, was more sensitive to diquat than it was to paraquat. Also, only paraquat cytotoxicity was found to be dependent on oxygen tension and could be altered by the presence of antioxidant enzymes in the culture medium. Both compounds, however, were found to be equipotent toward purified mitochondria. Both paraquat and diquat were able to uncouple oxidative phosphorylation and induce active oxygen species (superoxide anions and hydrogen peroxide) from this organelle. It is concluded that free-radical pathology is the most likely mechanism of action by which paraquat is cytotoxic toward these cells, but that diquat poisoning probably originates from some other mode of action.

INTRODUCTION

Paraquat (1,1′-dimethyl-4,4′-bipridylium) and diquat (1,1′-ethylene-2,2′-bipyridylium) are two stucturally similar herbicides that are used in agriculture today. Both compounds, though, also have been shown to be toxic to animals and humans (Bullivant, 1966; Brooks, 1971). Interesting, their respective target tissues are quite different. Paraquat initiates acute severe lung toxicity regardless of the route of exposure (Fisher et al., 1973; Vijeyaratnam and Corrin, 1971; Smith and Heath, 1974), whereas diquat produces acute gastrointestinal distress with only minimal pulmonary damage when taken orally (Cobb and Grimshaw, 1979; Crabtree et al., 1977).

This project was supported by a BRS grant, 2-S07-RR-05448-21, awarded to the University of Minnesota School of Public Health by the Biomedical Research Grant Program, Division of Research and Resources, National Institutes of Health.

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Journal of Toxicology and Environmental Health, 18:393–407, 1986

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From a toxicological point of view, this tissue specificity could originate from either or both of two possibilities: (1) a difference in the pharmacokinetic parameters between these two compounds once absorbed into the mammalian body, and/or (2) a difference in the relative sensitivity of various mammalian cells towards each of these two herbicides. This latter possibility could arise due to differences in uptake rates between the two herbicides, or if the two compounds affect different sites within the mammalian cell in order to produce their cytotoxicity.

Previous investigators have already shown that paraquat, but not diquat readily distributes to and bioconcentrates in lung tissue (Rose and Smith, 1977). Diquat, on the other hand, is only transiently present in the lung; it primarily distributes to the liver and kidneys (Rose et al., 1976). With respect to their suspected mechanisms of toxicity, both paraquat and diquat are thought to stimulate an excess production of active oxygen species within the mammalian cell (Youngman and Elstner, 1981; Winterbourn, 1981; Wong and Stevens, 1985; Witschi et al., 1977; Stancliffe and Pirie, 1971), and it is thought that this excess of active oxygen species ultimately leads to oxidative cell damage (Trush et al., 1981; Bus et al., 1980; Burk et al., 1980; Aldrich et al., 1983). This proposed mechanism is similar to that previously hypothesized for the phytotoxic action of these compounds (Calderbank, 1968) and originates from earlier findings that both compounds have been shown to be capable of undergoing an oxidation–reduction reaction in the presence of various cellular constituents in vitro with the concomitant formation of hydrogen peroxide, superoxide anions, and/or other active oxygen species (Gage, 1968; Baldwin et al., 1975; Farrington et al., 1973; Hassan and Fridovich, 1978).

With respect to the relative potency of these two herbicides toward specific mammalian cell types, no studies have ever been reported. Therefore, in the interest of evaluating further the tissue specificity of these two compounds, this study quantifies and compares the cytotoxicity of both paraquat and diquat toward a single lung cell type, the pulmonary alveolar macrophage. This cell type was chosen because it has been proposed that macrophage damage may be a contributing factor in the pathogenesis of pulmonary fibrosis with several environmental toxicants, including paraquat (Hill et al., 1982; Styles and Wilson, 1973; Wong and Stevens, 1985).

METHODS

Cell Isolation

Pulmonary alveolar macrophages (PAM) were harvested from adult, male Sprague-Dawley rats weighing between 200 and 225 g (Biolab;

Madison, Wis.) by bronchial lavage (Myrvik et al., 1961). Initially, anesthesia was produced by a 60-mg/kg pentobarbital ip injection. A tracheostomy was then performed. The lungs of each animal were lavaged 5 times with 5-ml aliquots of room-temperature Ca^{2+}- and Mg^{2+}-free Hanks balanced salt solution. The lavage fluid from each animal was pooled in a 50-ml conical centrifuge tube and centrifuged for 10 min at 1000 ×g. Each cell pellet was resuspended in Ham's F12 culture medium containing 10% (v/v) fetal calf serum at a concentration of 1–2 $\times 10^6$ cells/ml. The total number of viable cells in each preparation was assessed with the use of a hemocytometer (erythrosin B dye exclusion). The percent PAM in each cell preparation was determined optically after staining the cells with Wright–Giemsa stain. Only those cell preparations containing ≥95% PAM with ≥95% viability were utilized for these studies.

Cell Culture Conditions

Aliquots of the above cell suspension were diluted 1:10 with Ham's F12 culture medium containing 10% (v/v) fetal calf serum and then placed into clean, sterile Leighton tubes containing 9 × 36 mm glass coverslips. The cells were allowed to attach to the coverslips by incubating the tubes at 37°C for 1 h. After cell attachment the solution was decanted, and the cells were washed once with fresh medium. The exposure period began upon the addition of a 1-ml aliquot of prewarmed (37°C) medium (without serum) containing the desired level of herbicide to each Leighton tube. Each herbicide was added in the powdered form to the culture medium. Each tube was then sealed and placed into a CO_2 incubator and allowed to incubate for 8 h at 37°C. At the end of the exposure period, the coverslips were removed from the tubes, rinsed once with fresh Hanks balanced salt solution (room temperature), and then stained with erythrosin B to assess viability (live cells remain clear, dead cells become red). At least 200 cells per coverslip were counted, with the data expressed as percent viable cells. Two coverslips were utilized at each herbicide concentration, and at least four concentrations were used to describe a dose-response curve. At least three animals were used per experiment, with cells from a single animal used to produce a complete dose-response curve.

Herbicide Uptake Measurements

The absorption of ^{14}C-labeled herbicide into PAM was measured by a modified method of Wyllie (1979). Briefly, freshly isolated PAM were placed in suspension (10^6 cells/ml) in Ham's F12 culture medium, pH 7.4, containing 500 μM herbicide (5 μCi/ml). The cell suspension was then incubated at 37°C for up to 30 min. Immediately after the addition of the labeled herbicide and at each 10-min interval throughout the incubation period, aliquots were taken and centrifuged (1000 ×g; 5

min). The pelleted cells were resuspended and washed once with cold medium prior to sonication in Kontes microultrasonic cell disruptor. Each sonicate was then placed into a counting vial containing 10 ml Aquasol. Counting efficiency for ^{14}C was calculated at 58%, [^3H]Inulin was used simultaneously in the wash procedure to assess and subtract intercellular label.

Cell Respiration

Cell respiration was measured polarographically in a Gilson model 5/6 oxygraph equipped with a Clark-type electrode. For total cell respiration measurements, an aliquot of a cell suspension obtained after herbicide exposure ($1-2 \times 10^6$ cells/ml) was placed into the electrode chamber. After equilibration to 37°C, the chamber was sealed and the rate of O_2 utilization was recorded continuously for 5 min. The cell respiration rate was calculated from the slope of the linear portion of the data obtained and expressed as nanomoles O_2 per minute per 10^6 cells.

Mitochondrial Respiration

Liver mitochondria were isolated from adult rats by differential centrifugation in 50 m*M* Tris-MOPS buffer, pH 7.4, containing 0.25 *M* sucrose. Mitochondria were resuspended at 20–40 mg/ml, and an aliquot (0.2 ml) was added to the electrode chamber in the Gilson 5/6 oxygraph to start the respiration measurements. The electrode chamber initially contained 1.4 ml prewarmed (37°C) buffer: 100 m*M* KPO_4; 50 m*M* Tris-Cl, pH 7.5; 5% glucose; 10 m*M* glutamate; and 10 m*M* malate. O_2 uptake was then followed in order to measure state 4 respiration. Aliquots (12.5 μl) of 13 m*M* ADP were added to produce state 3 respiration. Respiration rates were calculated from the linear portions of the data obtained under each assay condition. Paraquat or diquat was dissolved in the electrode buffer at the desired concentrations just prior to use in these experiments.

Mitochondrial O_2^- and H_2O_2 Generation

The mitochondrial production of H_2O_2 was quantified by the method of Boveris et al. (1972). Isolated mitochondria (1–2 mg) were placed in 3 ml of the following reaction mixture: 225 m*M* mannitol; 75 m*M* sucrose; 30 m*M* Tris-MOPS, pH 7.4; 10 m*M* glutamate; 10 m*M* malate; and 0.15–0.2 mg purified cytochrome c peroxidase. The change in the optical density of the reaction mixture was then measured at 25°C in an Aminco DW-2A dual-beam spectrophotometer. An extinction coefficient of 55 mM^{-1} cm^{-1} was used to quantify the cytochrome c peroxidase–H_2O_2 complex (monitored at 424–404 nm). Hydrogen peroxide generation was always measured under state 4 respiration conditions. Cytochrome c peroxidase was purified from yeast by the method of Yonetani and Ray (1965).

The mitochondrial production of superoxide anions was measured as the superoxide dismutase-inhibitable reduction of nitrobluetetrazolium (NBT), according to the procedure of Beauchamp and Fridovich (1971). The reaction mixture contained 1–2 mg mitochondria in 3 ml of the reaction mixture already described plus 1 m*M* NBT. The reference cuvette additionally contained 1000 units purified superoxide dismutase (Sigma Chemical Co.). The reaction was followed spectrophotometrically at 25°C in an Aminco DW-2A dual beam spectrophotometer. An extinction coefficient of 1.09 $mM^{-1}cm^{-1}$ was used to quantify NBT reduction (monitored at 660 nm).

Protein Analysis

Protein quantification was accomplished by the method of Gornall et al. (1949). Briefly, an aliquot of the test sample (isolated mitochondria) was added to 0.1 ml of a 10% sodium deoxycholate solution. Distilled, deionized water was then added to bring the solution to a final volume of 1.5 ml. This suspension was then incubated for 30 min at 37°C. An aliquot (1.5 ml) of biuret reagent was then added to each tube, and the mixture was incubated another 10 min at room temperature. The absorbance of the solution was read at 540 nm in a Beckman DU-7HS spectrophotometer. Bovine serum albumin (0–.5 mg/assay) served as the standard.

Statistical Methods

A logit transformation of each normalized response value was made to linearize the dose-response curves obtained in each study. The "best fit" line for the data points was then determined using least-squares simple linear regression. The slope of the regression line was tested to be different from zero using analysis of variance (ANOVA) ($\alpha = 0.05$), and if the slope was significantly different from zero, both the LC50 value (or EC50 value) and the slope were recorded. In addition, the 95% confidence interval (C.I.) was calculated for the LC50 or EC50 value.

Statistical significance between dose-response curves was determined by comparing their mean response at a midpoint dose level by the use of a two-tailed *t*-test. Statistical significance between means was also evaluated using the two-tailed *t*-test.

RESULTS

As shown in Fig. 1, both paraquat and diquat are cytotoxic to adult rat pulmonary alveolar macrophages in vitro. An LC50 value of 0.94 m*M* (95% C.I. 0.79–1.12 m*M*) was calculated for paraquat when these cells

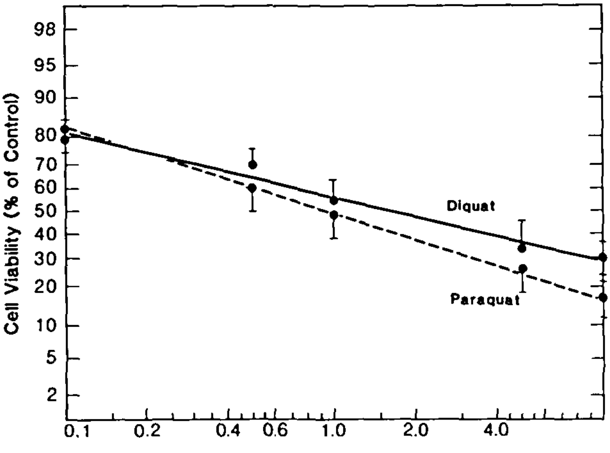


FIGURE 1. Dose-response curves of paraquat and diquat cytotoxicity in the pulmonary alveolar macrophage cell culture exposure system. Cells were exposed to each herbicide for 8 h in Ham's F12 culture medium without serum at 37°C. Cell viability was determined by dye exclusion (erythrosin B). Vertical bars indicate SE percentages ($n = 5$).

were exposed to this herbicide in primary culture for 8 h at 37°C. The LC_{50} value for diquat under the identical exposure conditions was 1.97 mM (95% C.I. 1.58–2.51 mM), statistically significant different from the paraquat value at the 5% level.

In an attempt to investigate this difference in cell sensitivity toward these two herbicides, uptake studies with ^{14}C-labeled paraquat and ^{14}C-labeled diquat were conducted. As shown in Fig. 2, diquat (the

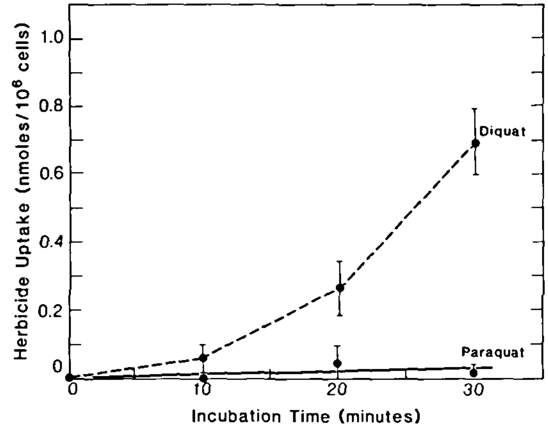


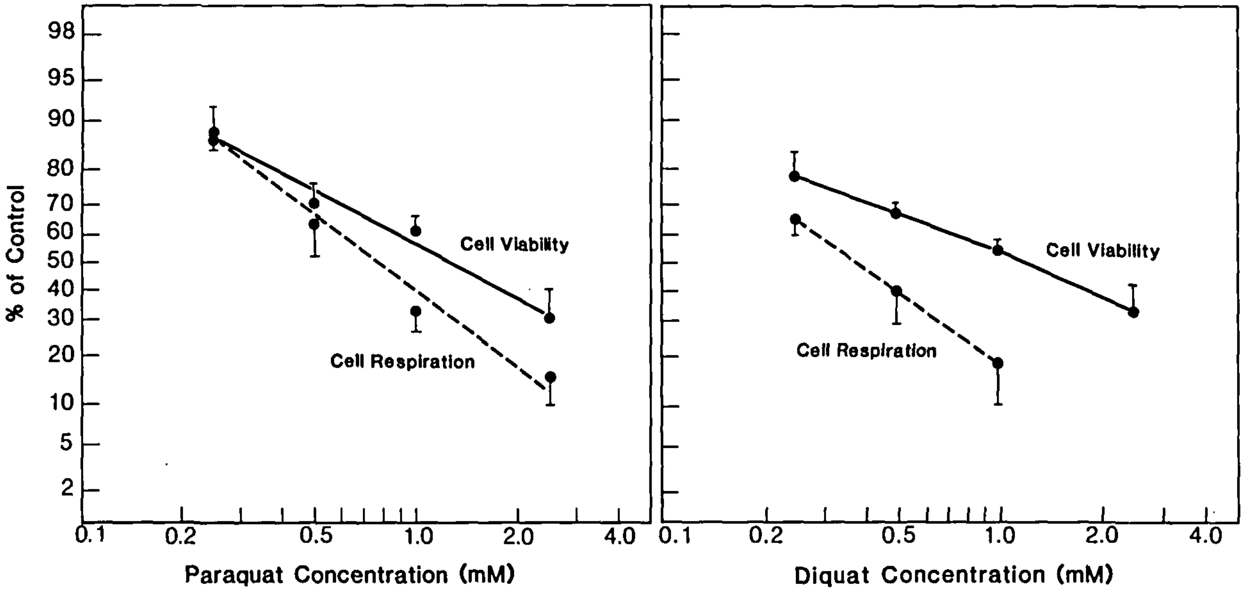
FIGURE 2. Herbicide uptake by adult rat pulmonary alveolar macrophages in vitro. Suspended cells (10^6/ml) were exposed to 500 μM herbicide in Ham's F12 culture medium without serum at 37°C. Vertical bars indicate SE ($n = 4$).

lesser of the two toxicants) was absorbed into these cells to a much greater extent than was paraquat. That the intracellular level of paraquat never did equal the level of diquat in these cells was determined by long-term (6-h) experiments (data not presented).

Therefore, one attractive interpretation that can be forwarded to explain these seemingly contradictory data is that each herbicide must be cytotoxic by a completely different mode of action. To investigate this possibility two types of experiments were conducted. In the first, the effects of oxygen tension and the presence of various antioxidant enzymes in the exposure system were evaluated. As described in Table 1A, equilibration of the culture media with 95% oxygen enhanced paraquat toxicity significantly but had no effect on the cytotoxicity of diquat. When incubated in air, 1 m*M* paraquat was lethal to approximately 50% of the cells in culture. When 95% oxygen was introduced into the exposure system the presence of 1 m*M* paraquat resulted in greater than 80% cell death. As can be seen, no such change in chemical potency occurred with diquat. The presence of superoxide dismutase at 1500 units/ml also only affected the paraquat-mediated cytotoxicity (Table 1B). Superoxide dismutase (SOD) significantly decreased the cytotoxicity of paraquat but not diquat in this culture system. Again, 1 m*M* paraquat was lethal to approximately 50% of these cells in culture. However, when SOD was added to the medium, less than 20% cell death was observed. Interestingly, the presence of catalase at 8200 units/ml had no effect on the cytotoxicity of either compound, and the combination of superoxide dismutase plus catalase in the culture media did not protect the cells additionally over that observed in the presence of superoxide dismutase alone.

In a second study designed to investigate possible differences in the mechanism of cytotoxicity of these herbicides, the effect of both compounds on alveolar macrophage respiration was followed (Fig. 3*a*, and *b*). As shown in Fig. 3*a*, cell respiration was a more sensitive index of paraquat poisoning than was cell death. An EC50 value for paraquat was determined to be 0.75 m*M* (95% C.I. 0.65–0.86 m*M*) when loss of O_2 consumption in the cell preparations was followed—statistically different from the LC50 value determined in this experiment, 1.12 m*M* (C.I. 1.01–1.63 m*M*).

When diquat was tested in a similar manner (Fig. 3*b*), cell respiration was again a more sensitive index of cytotoxicity than cell death, but the diquat-mediated loss in O_2 consumption exhibited a much steeper dose-response curve than did the paraquat-mediated response (Fig. 3*a*). The EC50 value for diquat using this index as a measurement of cell cytotoxicity was calculated to be 0.38 m*M* (95% C.I. 0.28–0.46 m*M*), again statistically significantly different from the LC50 value in this experiment, 1.52 m*M* (95% C.I. 1.22–1.89 m*M*), and from the comparable EC50 value for paraquat described above.



(*a*) (*b*)

FIGURE 3. Dose-response curves of paraquat and diquat cytotoxicity in the pulmonary alveolar macrophage cell culture exposure system. Exposure conditions were identical to those described in Fig. 1. Cell viability was measured by dye exclusion and cell respiration by O_2 consumption. Vertical bars indicate SE percentages ($n = 5$).

That the observed difference in mitochondrial sensitivity toward these two compounds was most likely due to differences in cell uptake of these two herbicides (Fig. 2) and not due to a difference in organelle sensitivity to both compounds was determined in the following investigation. In this experiment mitochondria were isolated from adult rat livers and then incubated in vitro in the presence of paraquat or diquat. Rat liver mitochondira were used in this experiment because a sufficient amount of macrophage mitochondria could not be obtained from a reasonable number of adult rats to conduct the experiment. Shown in Fig. 4*a* is the respiration pattern of a typical preparation of the mitochondria prepared in this investigation. As can be seen, when the mitochondria were added to the reaction buffer, a steady, linear rate of oxygen consumption was recorded (state 4 respiration). In a preliminary experiment it was shown that this rate, calculated as 4.2 ± 0.5 nmol O_2 consumed/min·mg mitochondrial protein (Table 2), was constant for up to 2 min in the assay system. When ADP was added to the preparation (arrow), state 3 respiration began. The average state 3/state 4 respiration ratio in these experiments was calculated at 5.3 ± 1.0, with the ADP/O in control mitochondira calculated as 2.7 ± 0.1.

When paraquat was present in the reaction mixture at a concentration of 1 μM, mitochondrial uncoupling resulted (Fig. 4*b*). As shown in Fig. 4*b*, in the presence of this herbicide, the usually linear state 4 respiration rate observed with unexposed mitochondria gradually increased with time until a very rapid O_2 consumption rate occurred. At

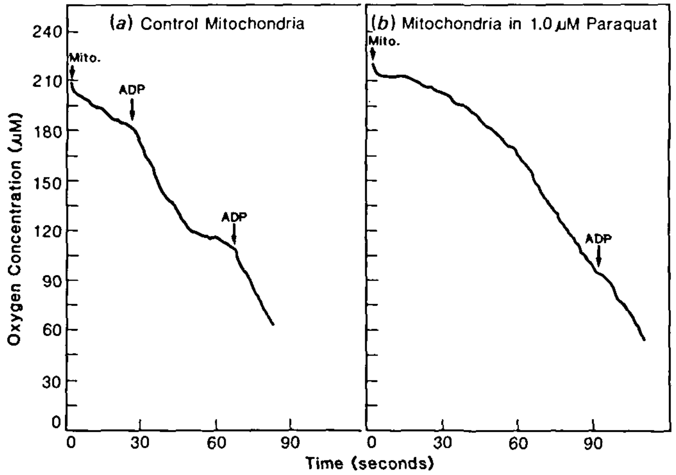


FIGURE 4. The effect of paraquat on rat liver mitochondrial respiration in vitro. Mitochondria were isolated and exposed to paraquat as described in the text. Figure represents tracings taken from Gilson 5/6 oxygraph. The oxygen concentration is expressed as 2 × O_2 concentration; ADP (162.5 nmol) was added (arrows) to initiate state 3 respiration. (a) Control mitochondria; (*b*) mitochondria in 1.0 μM paraquat.

this point, the addition of ADP to the reaction mixture (arrow) essentially had no effect. That this effect was dose-dependent with both herbicides can be seen in Table 2. As described in this table, increasing the concentration of either herbicide in the reaction mixture resulted in a drop in the ADP/O ratio (until no value could be calculated), a drop in the state 3/state 4 respiration ratio, and an increase in the state 4 respiration rate (as drawn from the final linear portion of the curve). In addition, the data provided in Table 2 show that isolated mitochondria are equally sensitive to both compounds.

Since the mitochondrial electron transport chain is known to be a potential source of active oxygen species especially when it is perturbed (Sata et al., 1983; Autor and Stevens, 1980), and since a previous experiment in this study (Table 1,A and B) suggests that paraquat is cytotoxic via active oxygen species, one last experiment was conducted to determine if these herbicides were able to stimulate superoxide anions and hydrogen peroxide from the mitochondrial electron transport chain. As shown in Table 3, both paraquat and diquat were able to enhance hydrogen peroxide and superoxide anion production from isolated mitochondria. Under the experimental conditions used in this study, which were similar to those that produced the uncoupling phenomenon observed in Fig. 4, paraquat was able to enhance H_2O_2 and O_2^- generation essentially threefold. In the presence of paraquat, H_2O_2

TABLE 1. Effect of Cell-Culture Conditions on Paraquat and Diquat Cytotoxicity in Vitro[a]

Herbicide	A. Oxygen tension, cell viability (% of control) Air (control)	95% O_2
1 m*M* Paraquat	52 ± 8	10 ± 9[b]
1 m*M* Diquat	63 ± 9	58 ± 7

Herbicide	B. Antioxidant enzymes, cell viability (% of control) Control	SOD	Cat	SOD + Cat
1 m*M* Paraquat	56 ± 6	82 ± 5[b]	60 ± 5	79 ± 6[b]
1 m*M* Diquat	59 ± 6	61 ± 8	64 ± 6	58 ± 5

[a] Cells were isolated, cultured, and exposed to either herbicide in an identical manner to that described in Fig. 1. Culture tubes were equilibrated with 95% oxygen just prior to their placement in the incubator and maintained in this environment throughout the 8-h exposure period. Superoxide dismutase (SOD) was present at 1500 units/ml and catalase (Cat) at 8200 units/ml. Data given as mean ± SD, as calculated from at least five determinations.

[b] Statistically significantly different from control value ($p < 0.05$).

generation increased from a basal level of 2.4 ± 0.6 nmol/min·mg protein to 6.7 ± 0.6 nmol/min·mg protein, and O_2^- production from 0.6 ± 0.1 nmol/min·mg protein to 1.6 ± 0.1 nmol/min·mg protein. Interestingly, diquat also similarly enhanced H_2O_2 and O_2^- generation. In these experiments H_2O_2 generation was increased from the basal rate of 2.9 ± 0.3 nmol/min·mg protein to 12.2 ± 1.2 nmol/min·mg protein, and O_2^- production from 0.6 ± 0.1 nmol/min·mg protein to 1.3 ± 0.2 nmol/min·mg protein.

DISCUSSION AND CONCLUSIONS

Under the in vitro exposure conditions employed in this study, both paraquat and diquat were found to be cytotoxic to adult rat alveolar macrophages. These two bipyridylium herbicides, however, did not exhibit equal potency toward these cells. Paraquat was statistically significantly more toxic to the alveolar macrophage than was diquat (Fig. 1), a finding that supports previous in vitro data that indicate that paraquat is the more potent pulmonary toxicant (Witschi et al., 1977; Rose and Smith, 1977). This is in contrast to data reported by Styles (1974), wherein both compounds were found to be equitoxic toward these cells. The exposure conditions described by Styles, however, result in a rapid loss in cell viability, whereas our conditions require much longer periods of exposure to show the cytotoxic effect and mimic more closely, at least with respect to herbicide concentration, conditions that occur in vivo (Rose et al., 1976). These longer exposure studies may allow better comparisons of potency between compounds.

In an attempt to explain our cell-sensitivity findings, the effect of both herbicides on various other biochemical parameters of cell function were investigated. It was shown, interestingly, that diquat was absorbed into these cells to a much greater extent than was paraquat (Fig. 2), a finding that seems contradictory to the cytotoxicity data. This seemingly contradictory data might be explained, at least in part, by previous findings in this laboratory (Wong and Stevens, 1985). In this earlier investigation it was reported that paraquat, but not diquat, was able to stimulate oxygen radical generation by alveolar macrophages by perturbing or interacting with an enzyme system on the plasma membrane surface. It was hypothesized at that time that perhaps the excess extracellular active oxygen species produced during this interaction might be the explanation for the cytotoxicity of this particular herbicide toward these cells. Indeed, data presented in this study (Table 1) further support this view. Paraquat cytotoxicity was shown to be enhanced by hyperoxic exposure conditions and reduced when the antioxidant enzyme superoxide dismutase was added to the cell culture medium. Since diquat was not found to interact with this surface enzyme in a similar manner, but as shown here was able to enter these cells to a much greater extent than was paraquat, another mode of action must be hypothesized for this herbicide. Since data in this study also show that diquat cytotoxicity was independent of oxygen tension and the presence of the antioxidant enzymes in the culture media (Table 1), it must be concluded that diquat cytotoxicity in our macrophage exposure system is not mediated by oxygen radicals or other active oxygen species. It was ascertained, however, that isolated mammalian mitochondria are sensitive to both diquat and paraquat, and that both herbicides were equally toxic to these organelles (Fig. 4 and Table 2). Both compounds were shown to uncouple the oxidative phosphorylation process, which is in agreement with previous investigators (Montgomery et al., 1982) who showed that mitochondria isolated from paraquat-treated lung slices were unresponsive to ADP. Yet in our whole-cell studies, diquat was definitely the more potent compound (Fig. 3). Diquat was capable of diminishing O_2 consumption at concentration levels well below those required of paraquat. This latter finding therefore is possibly due to the greater penetration rate of diquat into these cells (Fig. 2).

It was also shown that the presence of each herbicide was able to stimulate superoxide anion and hydrogen peroxide generation from isolated rat liver mitochondria (Table 3). This finding is in agreement with that of Sata et al. (1983), who showed that paraquat could enhance

TABLE 2. Paraquat and Diquat Effects[a] on Rat Liver Mitochondrial Respiration

Herbicide (μ*M*)	ADP/O	State 3/state 4	State 4 respiration (nmol/min · mg)
Paraquat			
0	2.7 ± 0.1	5.3 ± 1.0	4.2 ± 0.5
0.1	2.6 ± 0.2	4.8 ± 1.1	4.5 ± 0.6
0.25	1.9 ± 0.2[b]	3.0 ± 0.8[b]	6.5 ± 1.4[b]
0.5	1.5 ± 0.4[b]	2.1 ± 0.5[b]	10.8 ± 1.8[b]
1.0	—	1.3 ± 0.2[b]	12.8 ± 2.5[b]
2.5	—	1.1 ± 0.2[b]	16.4 ± 2.3[b]
10.0	—	1.02[c]	15.8[c]
Diquat			
0	2.8 ± 0.1	6.2 ± 0.8	3.8 ± 0.5
0.1	2.6 ± 0.2	4.6 ± 1.0[b]	4.4 ± 0.6
0.25	1.9 ± 0.2[b]	2.6 ± 0.3[b]	8.2 ± 0.8[b]
0.5	1.5 ± 0.3[b]	1.9 ± 0.3[b]	11.2 ± 1.6[b]
1.0	—	1.3 ± 0.2[b]	15.2 ± 3.3[b]
2.5	—	1.0 ± 0.1[b]	17.1 ± 2.8[b]
10.0	—	1.0[c]	16.4[c]

[a] Mean ± SE, as determined from three data points.

[b] *Statistically significantly different from the control (0 mM herbicide) value ($p < 0.05$).*

[c] Only one data point.

TABLE 3. Paraquat and Diquat Effects[a] on Mitochondrial O_2^- and H_2O_2 Generation

Herbicide (μM)	H_2O_2 generation (nmol/min · mg protein)	O_2^- generation (nmol/min · mg protein)
Paraquat		
0	2.4 ± 0.6	0.6 ± 0.1
1.0	6.7 ± 0.6[b]	1.6 ± 0.1[b]
Diquat		
0	2.9 ± 0.3	0.6 ± 0.1
1.0	12.2 ± 1.2[b]	1.3 ± 0.2[b]

[a] Mean ± SE, as determined from three data points.

[b] Statistically significantly different from the control value ($p <$ 0.05).

lipid peroxidation in submitochondrial particles. However, because an increase in oxygen tension and/or the presence of the antioxidant enzymes in the culture medium failed to alter the potency of diquat toward these cells (Table 1), it is concluded that these moderate increases in active oxygen species observed in the isolated mitochondria with both compounds either do not occur in intact cells or are insufficient in magnitude to initiate cell death via oxidative damage, i.e., lipid peroxidation. Since diquat is a potent uncoupler of oxidative phosphorylation in vitro and was shown to readily enter these cells, loss of mitochondrial function may be the mode of action by which this herbicide is cytotoxic to these cells. This hypothesis, however, awaits further study, as do others such as the NADPH depletion theory stated by Keeling and Smith (1982) and others (Suleiman and Stevens, 1985).

In conclusion, the pulmonary toxicity of paraquat as compared to diquat has been explained on the preferential retention in the lung of this herbicide (Rose and Smith, 1977). However, this hypothesis should now be modified since at concentration levels previously reported in the bloodstream of exposed animals (Rose et al., 1976) at least one lung cell type (and perhaps more) is now known to be more sensitive to paraquat than it is toward diquat.

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Received October 23, 1985
Accepted January 17, 1986